INTRACELLULAR CRYSTALLIZATION OF HEMOGLOBIN IN THE ERYTHROCYTES OF THE NORTHERN PIPEFISH, SYNGNATHUS FUSCUS

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It has long been recognized that the hemoglobins of different animals vary widely in solubility and ease of crystallization and that hemoglobin rarely crystallizes within the red blood corpuscles. In a previous paper (Dawson, 1930) intracellular crystallization of hemoglobin was described for the erythrocytes of the urodele, *Necturus maculosus*. In this case crystallization was apparently favored by previous poisoning with lead acetate, although it had been occasionally encountered in normal animals.

A similar phenomenon has been observed in the erythrocytes of the northern pipefish. The animals were obtained in the Eel Pond, at the Marine Biological Laboratory, Woods Hole, and appeared to be normal in all respects. The observation was made incidentally while studying supravitally the blood cells of the common marine fishes of that locality. The crystallization of hemoglobin in the erythrocytes of the pipefish is readily induced by slowly drying, in the air, rather thick smears of blood, and is most uniformly obtained when the humidity is relatively high. Preceding the appearance of definite crystals the cells lose their typical oval form and show an increasing tendency towards angularity. The majority finally assume a triangular shape but some become rhomboidal. However, at this stage the hemoglobin gives no evidence of crystal formation. Soon well-defined clefts appear in the cell contents and definite crystals then appear.

The number of crystals formed in individual cells is subject to some variation. Three crystals, forming the three sides of a triangle with the nucleus in the center, are most commonly encountered. Occasionally four crystals forming a rhomboidal figure, and more rarely two crystals arranged parallel with the long axis of the cell are present (Fig. 1). Frequently a variable number of very small, slender crystals may be associated with larger ones. They usually lie irregularly about the nucleus. The size of the larger crystals is also somewhat variable, but the shape is relatively constant. Practically all are

modified on the side next the cell membrane, being rounded rather than straight. In addition many are notched on the inner side especially if they are in contact with the surface of the nucleus.

Discussion

Little is known of the factors involved in maintaining the hemoglobin within the red blood cell in solution. In the present instance the only obvious cause of the crystallization of the hemoglobin in the

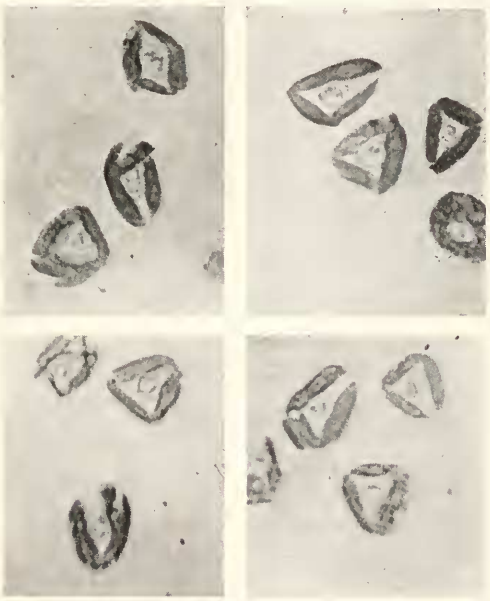

Fig. 1. Four selected areas from a preparation of pipefish blood showing the characteristic numbers, size, form, and position of hemoglobin crystals within the erythrocytes. The turbidity of the background is caused by the laking of many cells due to the injury produced in transferring the cells from the slide to a coverslip in order to obtain a preparation thin enough to photograph. Magnification × 1150.

erythrocytes of the pipefish is the slow withdrawal of water with whatever attendant injuries that may occur when drying takes place. It is of interest to note that the cell is deformed by the changed orientation of the hemoglobin molecules before any change in the nature of the hemoglobin can be observed with ordinary transmitted light and that the form acquired by the cell, triangular or rhomboidal, foreshadows the appearance of three or four major crystals within it. Moreover, the clefts which mark the amount of hemoglobin to be apportioned to each crystal also become evident while the hemoglobin still appears unmodified. One of the striking features of all erythro-

cytes is their tendency to return to their specific form after deformation, but in the case of incipient crystallization the shift in orientation of the hemoglobin molecules is sufficient to produce a permanent distortion.

In the case of *Necturus*, previously described, and in the pipefish the crystals of hemoglobin are large and relatively few. In other instances that have come under my observation while studying supravitally the erythrocytes of many vertebrates, the crystallization of hemoglobin has been quite different, the crystals being numerous and very small, producing a granular effect. Such crystallization has been encountered on a few occasions in *Necturus* as well as in another urodele, *Eurycea bislineata*. It has also been noted in several fishes such as the common mackerel, menhaden, alewife, and sea bass. In all of these cases the cause of the crystallization was unknown and appeared irregularly in preparations of fresh blood.

In a review of the literature one finds few references to intracellular crystallization of hemoglobin. Guerber (1927) observed it in the erythroblasts of embryos of the pig and cow. Kranz (1928) described crystals in mammalian erythrocytes after fixation with potassium bichromate and acetic acid, followed by paraffin imbedding. Celloidin imbedding gave negative results. He believed that the crystals were not pure hemoglobin but a product resulting from the reaction of hematin with the chromic and acetic acids. The work of Kranz was subsequently repeated by Tschachmachtschian (1932) who concluded that the crystals described by Kranz were entirely an artefact, the result of paraffin imbedding, and were not directly related to the hemoglobin content of the erythrocytes.

Jokl (1925), while studying fresh preparations of skate's blood, observed certain erythrocytes in which the cell content was divided obliquely by two or three peculiar light stripes. These light stripes appear comparable to the clefts which appear in the hemoglobin of the red cells of the pipefish, preceding the appearance of the large crystals.

Intracellular crystallization of hemoglobin was encountered in certain teleosts by Yoffey (1929), although he failed to recognize it as such. He states: "In the *Gadus* group the erythrocytes may assume a very curious shape. At first round, they then become oval, as in other fishes. They then show an increasing tendency towards angularity, and finally may become perfectly triangular in shape (Fig. 15). The relative proportion of triangular to oval red blood corpuscles varies from one animal to another. The illustration shown is from a blood film of *Gadus minutus* in which the majority of the erythrocytes

are triangular. On the other hand there are many cases in which only a few of the corpuscles are triangular, and the majority are of the normal shape. The triangularity is not artificially produced by the fixative because it may be observed in specimens of perfectly fresh and unfixed blood, though the angles may not be sharp as in the fixed film." (p. 336.)

From Yoffey's description it is obvious that in *Gadus minutus* he was dealing with intracellular crystallization and, in the photograph reproduced in his Fig. 15, three large crystals are clearly seen in almost every cell. Apparently in the *Gadus* group crystallization of hemoglobin occurs as readily as in the pipefish and was induced by Yoffey unconsciously by slight variations in his technique.

Summary

Crystallization of hemoglobin within the erythrocytes of the pipefish is described. This phenomenon is readily produced by slow drying, especially in a humid atmosphere.

Preceding the appearance of the definitive crystals the erythrocytes lose their characteristic oval form and become angular, triangular and rhomboidal forms predominating. Then definite clefts appear, followed soon after by the appearance of typical crystals. The number of crystals within individual cells varies. Two, three, and four large crystals are most commonly encountered, but a more variable number of minute needle-like forms may also be present in the erythrocyte.

It is of interest to note that the erythrocytes exhibit deformation due to the changing orientation of the hemoglobin molecules before any evidence of crystal formation can be detected with ordinary transmitted light.

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